Reference: *Biol. Bull.* 173: 178–187. (August, 1987)

CYTOCHEMICAL FEATURES OF SHRIMP HEMOCYTES

JO ELLEN HOSE, GARY G. MARTIN, VAN ANH NGUYEN, JOHN LUCAS, AND TEDD ROSENSTEIN

Department of Biology, Occidental College, Los Angeles, California 90041

Abstract

Morphological studies suggest that there are several types of decapod hemocytes; however, distinguishing criteria based on conventional staining techniques are often subtle or ambiguous. Cytochemical features of ridgeback prawn (Penaeidae: *Sicyonia ingentis*) hemocytes were studied using specific stains for lysosomes, cytoplasmic contents, and granule enzymes. This approach facilitates the differentiation of cell types in the ridgeback prawn and provides information on the functions of and relationships among different cell types.

Agranular hemocytes and a subgroup of small granule hemocytes contain extensive cytoplasmic glycoprotein deposits which display smudgy, intense staining with Sudan black B. As previously shown, coagulogen—the clotting material in decapods—stains with Sudan black B when extracted from lysed hemocytes. Other hemocyte types display light staining limited to granule membranes.

Lysosomes are not observed in agranular cells and are rarely present in small granule hemocytes with glycoprotein deposits. Small granule hemocytes without deposits and large granule hemocytes contain numerous lysosomes as shown by the presence of acid phosphatase, β-glucuronidase, and nonspecific esterase. Acid phosphatase is observed in the Golgi body of these cells, within small vesicles, and in small granules. The granules in large granule hemocytes rarely show acid phosphatase reaction, yet small acid phosphatase-positive vesicles fuse with the large granules. The acid phosphatase in the large granules may exist in an inactive form. Prophenoloxidase activity is localized only in large granules. The physiological significance of hemocyte cytochemistry is also discussed.

Introduction

Crustacean hemocytes perform a variety of physiological and pathological functions including coagulation (Ravindranath, 1980), phagocytosis, recognition of foreign material, carbohydrate transport, and encapsulation (Bauchau, 1981). Several hemocyte categories have been recognized in decapod crustaceans based on morphological criteria. Morphological features are often subtle and ambiguous, and are not readily recognized by other investigators. In addition, morphological criteria are rarely based on properties that facilitate the differentiation between stages in hemocyte maturation or among cells with different physiological functions. Hence, previous investigations have failed to define a clear correspondence between various cell types and their functions.

In an attempt to develop a comprehensive description of crustacean hemocyte formation and function, cytochemical techniques were used to complement our previous morphological description of shrimp hematopoietic tissue (Martin *et al.*, 1987) and circulating hemocytes (Martin and Graves, 1985). Electron microscopic exami-

Received 2 January 1987; accepted 19 May 1987.

nation of hemocytes from the ridgeback prawn (Penaeidae: *Sicyonia ingentis*) shows the existence of four cell types: agranular, small granule with cytoplasmic deposits, small granule without cytoplasmic deposits, and large granule hemocytes. Agranular hemocytes are small cells with a high nucleus:cytoplasm ratio (Martin *et al.*, 1987). Their cytoplasm contains little other than aggregations of electron-dense deposits. A subset of small granule hemocytes contains similar electron-dense cytoplasmic deposits, one to six round striated granules, and occasional electron-dense granules. In contrast, a distinct subset of small granule hemocytes and the large granule hemocytes lack cytoplasmic deposits and striated granules. These hemocytes have many (>10) electron-dense, electron-lucent, or punctate granules which range in diameter from 0.4 μm in small granule hemocytes to 0.8 μm in large granule cells. Intermediate stages were observed between agranular hemocytes and small granule hemocytes with deposits and between small granule hemocytes without deposits and large granule hemocytes, suggesting the existence of two distinct hemocyte lines.

In view of the difficulty in accurately identifying certain hemocyte categories at the light microscope level, various enzymatic and cytochemical methods were evaluated for use in hemocyte classification. The goals of this study are to (1) identify cytochemical stains which can be used to differentiate specific hemocyte types, and (2) provide useful information on the function of the various cell types.

Materials and Methods

Animals

Ridgeback prawns were collected and maintained as previously described (Martin *et al.*, 1987). Shrimp averaged 14.5 g and were in molt stages C and D (Anderson, 1985).

Tissue collection and preparation

Hemolymph (usually 0.2 cc) was withdrawn from the ventral sinus or heart into a 1 cc syringe containing anticoagulant (Martin and Graves, 1985). Hemocyte smears were then prepared on glass microscope slides, allowed to air dry, and used for light microscopy.

Hemocytes and hematopoietic nodules to be examined at the electron microscopic (EM) level were fixed in 2.5% glutaraldehyde in 0.1 *M* sodium cacodylate (pH 7.8) containing 12% glucose for 1 h at room temperature. Following a 30 min wash in 0.1 *M* sodium cacodylate (pH 7.8) containing 24% sucrose, the tissues were post-fixed in 1% OsO_4 in 0.1 *M* sodium cacodylate for 1 h at room temperature, dehydrated in a graded series of ethanol, and infiltrated and embedded in Spurrs' (1969) low viscosity plastic.

Epigastric hematopoietic nodules were dissected from shrimp as described by Martin *et al.* (1987). Touch preparations of sagittally cut nodules were air-dried prior to the cytochemical demonstration of prophenoloxidase (Ppo). Frozen sections (7 μm thick) were cut using a Tissue Tek II cryostat for the demonstration of lysosomal enzymes. Thin sections (7 μm) were also prepared using formalin-fixed, paraffin-embedded tissue.

Demonstration of cytoplasmic constituents

Following a two-minute fixation in absolute ethanol, smears were stained with bromphenol blue or periodic acid-Schiff (PAS). Smears to be stained with Best's car-

mine (Sheehan and Hrapchak, 1980) were fixed in ethanol for 30 minutes. PAS and carmine were compared with and without prior digestion by α-amylase. For the digestions, hemocytes were suspended in 0.5% aqueous amylase for 1 h, then pelleted by a 5 min centrifugation at $500 \times g$ in a table top centrifuge before preparation of the smear. Sections of hematopoietic nodule were stained with bromphenol blue or PAS.

Enzymatic extractions to demonstrate composition of cytoplasmic deposits and granules were also examined using EM. Hemocytes were fixed in 2.5% glutaraldehyde in 0.1 *M* sodium cacodylate (pH 7.8) containing 12% glucose for 1 h, then washed in cacodylate buffer and kept at 4°C for 12 to 18 h. The hemocyte pellet was then dehydrated through a graded ethanol series, infiltrated and embedded in Spurrs' (1969) low viscosity plastic. Thin sections were cut on a Porter Blum MT2B ultramicrotome, picked up on gold grids, and floated on one of the following solutions for 2 to 20 h at 37°C: (A) 0.5% protease in 0.2 *M* phosphate buffer (pH 7.4) or (B) 0.5% α-amylase in 0.2 *M* phosphate buffer (pH 7.4). These sections and control sections (floated on distilled water for an equivalent period of time) were examined unstained and stained (0.5% uranyl acetate in 0.05 *M* Tris-maleate for 1 h at room temperature) using a Hitachi HU11A transmission electron microscope.

Lipids were demonstrated in hemocyte smears and nodule touch preparations using a commercial Sudan black B kit (Sigma Chemical Co. Kit #380) according to provided directions.

Prophenoloxidase activity

To test for the presence of prophenoloxidase (Ppo), hemocytes and hematopoietic tissue touch preparations were fixed in 2.5% glutaraldehyde in 0.1 *M* phosphate buffer (pH 7.4) for 1 h at 4°C. The cells were given three 15-min rinses in phosphate buffer, incubated in 0.1% L-DOPA in phosphate buffer for 16 h at room temperature (Soderhall and Smith, 1976), and examined by light microscopy.

Lysosomal enzymes

The presence of acid phosphatase (Sigma Chemical Co. Kit #386), β-glucuronidase (Kit #180), and α-aryl naphthyl esterase—a nonspecific esterase (Kit #90)—were demonstrated at the light microscopic level using commercial research kits (Sigma Chemical Co.). Hemocyte smears and frozen sections of hematopoietic nodule were fixed in glutaraldehyde and incubated according to provided directions. Staining patterns for each enzyme were quantified at 1000× by estimating the numbers and sizes of positive areas in 10 cells from each of the 4 hemocyte categories described by Martin *et al.* (1987).

To localize acid phosphatase at the EM level, fixed hemocytes and hematopoietic nodules were washed thoroughly and then incubated in a medium consisting of 40 m*M* Tris-maleate buffer (pH 5), 11.5 m*M* sodium β-glycerophosphate, 2.4 m*M* lead nitrate, and 5% sucrose at 37°C for 2 h. Hemocytes were then processed as described above.

The same procedure was followed for glucose-6-phosphatase, alkaline phosphatase, and peroxidase except for the use of different incubation media. For glucose-6-phosphatase, the fixed cells were incubated in a medium composed of 25 mg glucose-6-phosphate, 27 ml distilled water, and 20 ml of 0.3 *M* Tris-maleate buffer (pH 9.7). The incubation medium for alkaline phosphatase consisted of 4 ml 1.25% sodium β-glycerophosphate, 4 ml of 0.2 *M* Tris-maleate buffer (pH 9), 9.4 ml distilled water, and 2.6 ml of 1% lead nitrate. The peroxidase medium contained 5 mg 3,3-diamino-

benzidine tetrahydrochloride, 10 ml of Tris-maleate buffer (pH 7.6), and 0.1 ml of 1% H_2O_2.

Results

Cytoplasmic constituents

The abundant cytoplasmic deposits of agranular hemocytes and a subset of small granule hemocytes are composed of glycoproteins as evidenced by positive reactions with PAS, carmine, and bromphenol blue. Digestion with α-amylase prior to application of PAS and carmine reduced but did not completely remove the staining of these cytoplasmic deposits. Tissue sections of the epigastric hematopoietic nodule stained with PAS or bromphenol blue yields results similar to those in free hemocytes.

Sudan black B produces a distinctive staining pattern in agranular hemocytes and small granule hemocytes with cytoplasmic deposits (Figs. 1, 2). These cells appear smudgy, with the heavy dark stain obscuring nuclear characteristics. Only a thin clear zone adjacent to the plasma membrane is occasionally present. Staining of the cytoplasmic deposits by Sudan black B indicates the presence of a lipid moiety associated with the glycoprotein. In small granule hemocytes lacking deposits (Fig. 3) and in large granule hemocytes (Fig. 4), delicate staining is evident only around granule and nuclear membranes, producing a diffuse pattern. Maturing hemocytes from the hematopoietic nodule display identical staining patterns.

Granule histochemistry

Granules in free and maturing hemocytes are stained with PAS, carmine, and bromphenol blue, indicating the presence of glycoproteins. Prior amylase digestion removes granular staining by PAS and carmine.

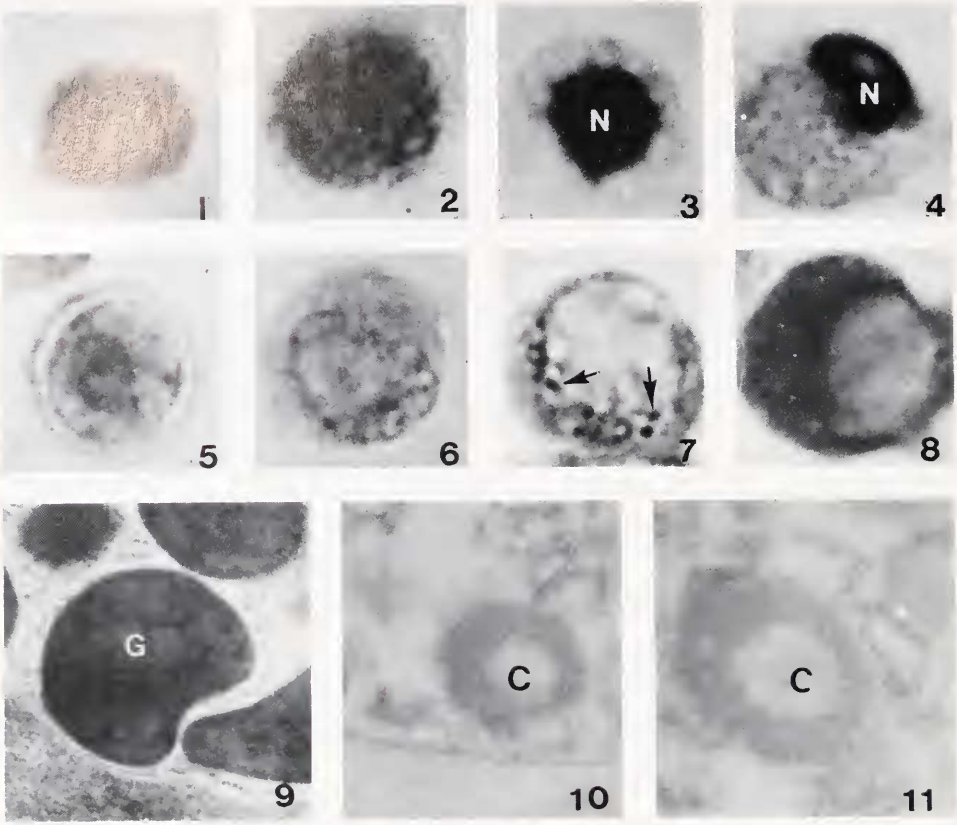
Prophenoloxidase activity is visualized following incubations of fixed hemocytes and hematopoietic tissue touch preparations with L-DOPA (Figs. 5–8). Ppo activity is limited to granules of small granule hemocytes lacking glycoprotein deposits and large granule hemocytes. In some animals (molt stage D), almost 100% of these cell types display intense activity (>10 positive granules each) while in intermolt shrimp, less than 1% of these cells are positive. Similar results are obtained using hemocyte smears and tissue touch preparations.

No peroxidase activity is observed in any of the hemocyte categories.

The glycoprotein content of large and small granules is also seen in sectioned tissues that were subsequently treated with protease or α-amylase. Figure 9 shows a large granule from a hemocyte viewed after standard preparation. Figures 10 and 11 show granules in sections treated with α-amylase (12 h) and protease (6 h). At these times, the core of the granules has been extracted, however, with longer incubations (20 h), the entire granule is extracted by both enzymes.

Lysosomal enzymes

Three hydrolases (acid phosphatase, β-glucuronidase, and nonspecific esterase) were used to demonstrate the presence of lysosomes in hemocytes at the light microscope level (Table I). These stains yield similar cytochemical information for each specific hemocyte type although individual hydrolases produce slightly different staining patterns. Agranular hemocytes do not contain any of the lysosomal enzymes. Glycoprotein-rich small granule hemocytes exhibit between zero and three focally positive areas consistent with the size of lysosomes. These cells occasionally contain



FIGURES 1–4. Light micrographs of agranular, small granule hemocyte with deposits, small granule hemocyte without deposits, and large granule hemocyte, respectively, treated to show sites of prophenoloxidase. The first two cells have no reaction product. The granules (arrows) in the small granule hemocyte without deposits react as does the entire cytoplasm of the large granule hemocyte. All figures 2500×.

FIGURES 5–8. Light micrographs of same cell types as in Figures 1–4, treated with Sudan black B. Agranular and small granule hemocytes with deposits show dense reaction products in the cytoplasm which obscure the nucleus. The latter two cell types have minimal staining and the nucleus (N) is clearly observed. All figures 2500×.

FIGURE 9. Transmission electron micrograph showing homogeneous and electron-dense granules (G) from a large granule hemocyte fixed with both glutaraldehyde and osmium and stained with uranyl acetate and lead citrate. 43,000×.

FIGURE 10. Transmission electron micrograph showing a granule from a large granule hemocyte that was fixed only with glutaraldehyde. Thin sections were floated on a protease solution for 6 h and examined without stain. Note the low electron density and extraction of the granule core (C). 43,000×.

FIGURE 11. Transmission electron micrograph of a granule from a large granule hemocyte prepared as in Figure 10 and then floated on a solution of α-amylase for 12 h. Note the low electron density of the granule and extraction of its core (C). 43,000×.

a few acid phosphatase-positive granules as well. In contrast, small granule hemocytes lacking cytoplasmic deposits have from three to eight positive foci consistent with lysosomes. Half of these cells have only a few (0–3) positive granules while the remaining small hemocytes contain over 30 positive granules. A few of the latter group, presumably transitional to large granule hemocytes, also exhibit a few large acid phosphatase-positive granules. In the large granule hemocytes, up to three focally positive

TABLE I

Distribution of lysosomal enzymes in shrimp hemocytes

Hemocyte type	Acid phosphatase	β-glucuronidase	Glucose-6-phosphatase	Non-specific esterase	Alkaline phosphatase
Agranular	None	None	None	None	None
Small granule hemocyte with deposits	Rare (1–3 RS*/ Cell)	Rare (1–3 RS/ Cell)	None	Few (1–10 RS/ Cell)	None
Small granule hemocyte without deposits	Mixed (50% of cells have >30 RS/ Cell 50% of cells have 1–10 RS/ Cell)	Many (>10 RS/Cell)	None	Intermediate (10–30 RS/Cell)	None
Large granule hemocytes	Mixed (50% of cells have 0–1 RS/Cell and nuclei are pycnotic 50% of cells have 4–8 RS/Cell)	Many (>10 RS/Cell)	None	Many (>30 RS/Cell)	None

* RS stands for reaction sites.

areas consistent with lysosomes were observed. From zero to five small granules are positive as well as from zero to two large granules. Among the large granule hemocytes, the largest cells which contain eccentrically placed, pycnotic nuclei were usually acid phosphatase-negative or contain only one positive focus.

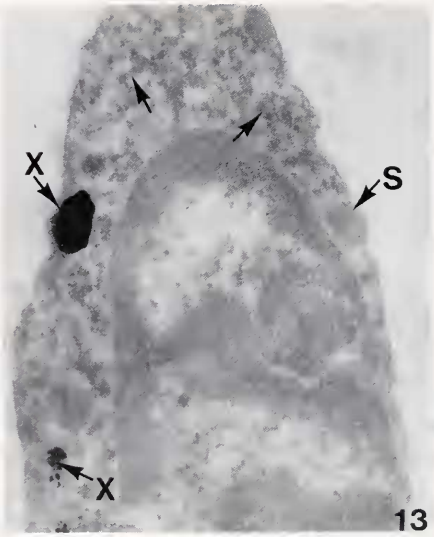
Electron microscopy localization of acid phosphatase yields similar results with no reaction product detected in agranular hemocyte (Fig. 12). Staining is infrequently observed in small granule hemocytes containing glycoprotein deposits and is restricted to small vesicles and granules of the non-striated variety (Fig. 13). Heavy staining is found in the granules of the small granule hemocyte lacking deposits (Fig. 14). Large granule hemocytes have reaction product dispersed throughout the cell in vesicles and the smaller granules. Only a few of the large granules stain positive although these were morphologically indistinguishable from non-reactive granules (Fig. 15). In Figure 16, acid phosphatase-positive trans cisternae and small vesicles are shown budding from a Golgi body. These vesicles (Fig. 16, inset) appear to progressively coalesce, forming larger reaction vesicles (Fig. 17), then small granules, and finally large granules (Fig. 18).

Nonspecific esterase is observed only in granulated cells. Glycoprotein-rich small granule hemocytes are completely negative or contain up to 10 tiny positive areas consistent with the size of vesicles. In contrast, from 10 to over 30 positive vesicles are observed in small granule hemocytes without cytoplasmic deposits. Large granule hemocytes contain numerous (>30) positive vesicles. Patterns of β-glucuronidase staining in granulated cells are similar to those of nonspecific esterase in addition to the presence of a few (<3) positive foci of lysosomal size in the small granule hemocytes.

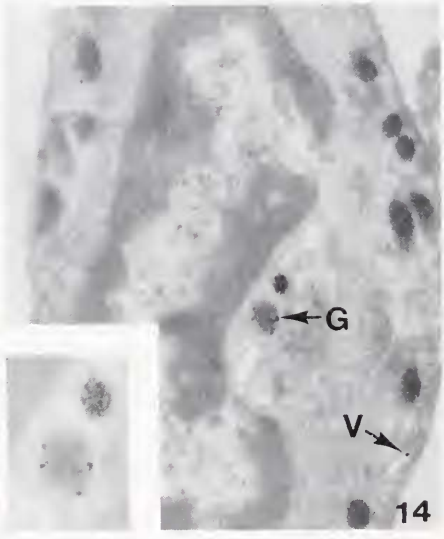
Maturing hemocytes from frozen sections of the hematopoietic nodule were examined for the presence of acid phosphatase and β-glucuronidase. Staining patterns of acid phosphatase are identical between maturing hemocytes and those described above for free hemocytes. β-glucuronidase activity is not observed in touch preparations of the hematopoietic nodule.

All hemocytes are negative for alkaline phosphatase and glucose-6-phosphatase.

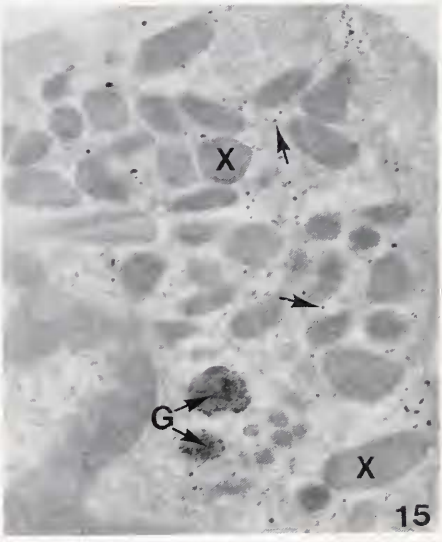
12



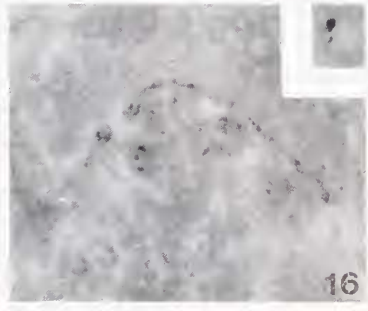
13



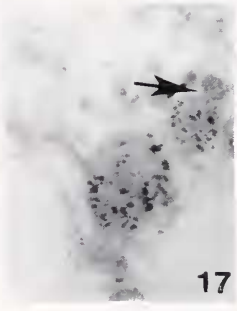
14



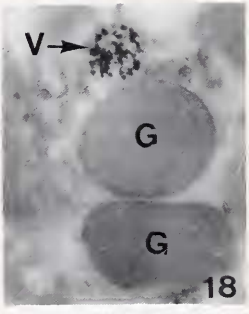
15



16



17



18

Discussion

Results of cytochemical tests support the morphological classification of ridgeback prawn hemocytes previously developed in our laboratory (Martin *et al.*, 1987) and yield information on the physiological functions performed by the various hemocyte types. A combination of two or three cytochemical tests is suggested for classification of shrimp hemocytes. Sudan black B produces a distinctive smudgy staining pattern in agranular hemocytes and small granule hemocytes with cytoplasmic deposits. Acid phosphatase can be used to differentiate agranular cells, which are negative for lysosomal enzymes. Prophenoloxidase activity is limited to small granule hemocytes without cytoplasmic deposits and large granule hemocytes; however, significant activity may only be demonstrable during the D stage of the molt cycle (Bauchau, 1981).

The glycoprotein deposits in the cytoplasm of agranular hemocytes and a subgroup of small granule hemocytes are distributed in linear arrays throughout the entire cell and are evident in all molt stages (unpub. obs.). In contrast, glycogen—which it resembles ultrastructurally—is typically confined to one area of decapod hemocytes and does not have a linear arrangement (Johnston *et al.*, 1973; Bauchau, 1981). Glycogen has been shown to be transported by hemocytes (Johnston *et al.*, 1973; Bauchau, 1981) and may involve the enzyme glucose-6-phosphatase (Johnston and Davies, 1972). This enzyme, however, was not detected in shrimp hemocytes. The glycoprotein may contain a lipid moiety since the deposits are intensely stained by Sudan black B. Such chemical properties are consistent with those of the primary coagulation protein, coagulogen (Durliat, 1985). In decapod hemocytes, intracellular coagulogen does not appear to be localized in granules although granules are necessary for coagulation to occur (Ravindranath, 1980; Durliat, 1985). Shrimp small granule hemocytes with lipoglycoprotein deposits contain granules with a striated or concentric substructure (Martin *et al.*, 1987). Similar granules have been observed in *Limulus* (Copeland and Levin, 1985), crabs (Bodammer, 1978), lobsters (Hearing and Vernick, 1967; Goldenberg *et al.*, 1986), and crayfish (Unestam and Nylund, 1972), and alterations in the striated granules of shrimp have been observed early in the process of hemolymph coagulation (unpub. obs.).

Lysosomes were observed in each cell type except for agranular hemocytes. Small

---

Figures 12 and 13. Transmission electron micrographs of an agranular hemocyte (Fig. 12) and a small granule hemocyte with deposits (Fig. 13) treated to display sites of acid phosphatase activity. No reaction sites are present in agranular cells. In small granule hemocytes with deposits, reaction sites (×) are rare and then localized to granules of the electron-dense variety. Striated granules (S) are never labelled. In both cells, note the small amount of cytoplasm which contains the deposits (arrows). Both figures 20,000×.

Figure 14. Transmission electron micrograph of a small granule hemocyte that lacks deposits showing a few reaction sites for acid phosphatase in granules (G) and vesicles (V). 20,000×. Inset shows a higher magnification (43,000×) micrograph of the small granules.

Figure 15. Transmission electron micrograph of a large granule hemocyte showing reaction sites for acid phosphatase in vesicles throughout the cytoplasm (arrows) and some of the granules (G). Other granules (×) show no reaction product. 20,000×.

Figure 16. Transmission electron micrograph showing a Golgi body in a large granule hemocyte. The trans-cisternae contains the acid phosphatase reaction product. 42,000×. Inset shows a vesicle with reaction product. Similar vesicles are commonly seen around Golgi bodies as well as throughout the cytoplasm. 42,000×.

Figure 17. Transmission electron micrograph showing acid phosphatase reaction product in three vesicles of increasing diameter. Note how the smallest vesicle appears to be fusing (arrow) with the medium sized vesicle and that the contents of the vesicles are not as electron dense as fully mature granules. 32,000×.

Figure 18. Transmission electron micrograph showing acid phosphatase reaction product in one large vesicle (V) and not in two adjacent granules (G). 32,000×.

granule hemocytes with glycoprotein deposits contained only one to three lysosomes per cell. In small granule hemocytes without deposits and in large granule hemocytes, many lysosomes were identified using LM and EM cytochemistry. Although the granules in these cells are morphologically indistinguishable, they may be enzymatically heterogeneous (see Bauchau, 1981). Using TEM, acid phosphatase was localized in some but not all of the granules of the shrimp. The same results were observed in granulocytes of the clam *Mercenaria mercenaria* (Yoshino and Cheng, 1976) and interpreted to indicate a heterogeneous population of granules or a non-synchronized cycle of granule production, perhaps with the final enzyme stored in an inactive form. The presence of numerous lysosomes in the large and small granulocytes which lack deposits supports the suggestion that these cells are phagocytic (Bauchau, 1981; Soderhall *et al.*, 1986) and that the granules are available for intracellular degradation processes. However, because of the large number of granules in a single hemocyte, it is unlikely that granules could be exclusively reserved for phagocytosis. Other researchers suggested extracellular functions for these granules, including recognition of foreign material (Soderhall and Smith, 1983) and agglutinin sequestration (Stang-Voss, 1971).

The recognition of foreign material in arthropods is mediated by the prophenoloxidase system which is located in the granules (Soderhall and Smith, 1983). Results of the present study show that only large granule hemocytes and small granule hemocytes without deposits contain prophenoloxidase activity. Soderhall and Smith (1983) obtained similar conclusions of Ppo activity within granular hemocytes in the crab, *Carcinus maenus*. Ppo activation and exocytosis in response to endotoxin or β-glucan exposure can initiate the coagulation cascade and serves as the crustacean equivalent to the alternate complement and properdin pathways in mammals (Durliat, 1985). The proposed existence of the Ppo system in the small granule hemocytes without deposits–large granule hemocyte line and the coagulation enzymes in the other hemocyte line suggests cooperativity among shrimp hemocytes during endotoxin-mediated coagulation analogous to that observed for defense reactions in insects (Ratcliffe *et al.*, 1984) and crustaceans (Soderhall *et al.*, 1986).

Based upon results of morphological (Martin *et al.*, 1987) and cytochemical studies, shrimp hemocytes can be divided into two cell lines, the deposit line (composed of agranular and striated granule hemocytes) and the granulocyte line (small and large granule hemocytes). Although the lysosomal enzyme data presented here are consistent with maturing stages of a single cell line, the following arguments support our theory: (1) striated granules are never found in granulocytes (The striated granule shown in Fig. 7B of Martin and Graves, 1985, was taken from a small granule hemocyte containing cytoplasmic deposits. However, at that time, no distinction was made between the deposit and granulocyte lines.); (2) glycolipoprotein deposits are never observed in granulocytes; (3) mitosis is observed both in agranular hemocytes and in small granule hemocytes which lack cytoplasmic deposits; (4) cells are present as a continuum of differentiation between agranular and striated granule hemocytes and between small and large granule hemocytes; and (5) clusters of deposit cells and granulocytes are usually segregated within the hematopoietic tissue. The utility of this classification scheme must now be determined by functional studies identifying the role of the various hemocyte types in crucial biological processes such as coagulation, defense reactions, wound healing, and exoskeleton hardening.

Acknowledgments

We thank Terri Fu and Sidne Omori for their technical help. The project was supported by NSF grant DCB-8502150 to GM and JEH.

LITERATURE CITED

ANDERSON, S. L. 1985. Multiple spawning and molt synchrony in a free spawning shrimp (*Sicyonia ingentis:* Penaeoidea). *Biol. Bull.* **168:** 377–394.

BAUCHAU, A. G. 1981. Crustaceans. Pp 386–420 in *Invertebrate Blood Cells,* Vol. 2. Academic Press, New York.

BODAMMER, J. E. 1978. Cytological observations on the blood and hemopoietic tissue in the crab, *Callinectes sapidus.* I. The fine structure of hemocytes from intermoult animals. *Cell. Tissue Res.* **187:** 79–86.

COPELAND, D. E., AND J. LEVIN. 1985. The fine structure of the amoebocyte in the blood of *Limulus polyphemus.* I. Morphology of the normal cell. *Biol. Bull.* **169:** 449–457.

DURLIAT, M. 1985. Clotting processes in Crustacea Decapoda. *Biol. Rev.* **60:** 473–498.

GOLDENBERG, P. Z., A. H. GREENBERG, AND J. M. GERRARD. 1986. Activation of lobster hemocytes: cytoarchitectural aspects. *J. Invertebr. Pathol.* **47:** 143–154.

HEARING, V. J., AND S. H. VERNICK. 1967. Fine structure of the blood cells of the lobster, *Homarus americanus. Ches. Sci.* **8:** 170.

JOHNSTON, M. A., AND P. S. DAVIES. 1972. Carbohydrates of the hepatopancreas and blood tissues of *Carcinus. Comp. Biochem. Physiol.* **41B:** 433–443.

JOHNSTON, M. A., H. Y. ELDER, AND P. S. DAVIES. 1983. Cytology of *Carcinus* haemocytes and their function in carbohydrate metabolism. *Comp. Biochem. Physiol.* **46A:** 569–581.

MARTIN, G. G., AND B. L. GRAVES. 1985. Fine structure and classification of shrimp hemocytes. *J. Morphol.* **185:** 339–348.

MARTIN, G. G., J. E. HOSE, AND J. J. KIM. 1987. Structure of hematopoietic nodules in the ridgeback prawn, *Sicyonia ingentis:* light and electron microscopic observations. *J. Morphol.* **192:** 193–204.

RATCLIFFE, N. A., C. LEONARD, AND A. F. ROWLEY. 1984. Prophenoloxidase activation: nonself recognition and cell cooperation in insect immunity. *Science* **226:** 557–559.

RAVINDRANATH, M. H. 1980. Haemocytes in haemolymph coagulation of arthropods. *Biol. Rev.* **55:** 139–170.

SHEEHAN, D. C., AND B. B. HRAPCHAK. 1980. *Theory and Practice of Histotechnology.* C. V. Mosby Co., St. Louis. 481 pp.

SODERHALL, K., AND V. J. SMITH. 1983. Separation of haemocyte populations of *Carcinus maenus* and other marine decapods, and prophenoloxidase distribution. *Dev. Comp. Immunol.* **7:** 229–239.

SODERHALL, K., V. J. SMITH, AND M. W. JOHANSSON. 1986. Exocytosis and uptake of bacteria by isolated haemocyte populations of two crustaceas: evidence for cellular co-operation in the defence reactions of arthropods. *Cell Tissue Res.* **245:** 43–49.

SPURRS, A. 1969. A low viscosity epoxy embedding medium for electron microscopy. *J. Ultrastruct. Res.* **26:** 31–43.

STANG-VOSS, C. 1971. Zur ultrastruktur der blutzellen wirbelloser tiere. V. Uber die hamocyten von *Astacus astacus* (L.) (Crustacea). *Z. Zellforsch.* **122:** 68–75.

UNESTAM, T., AND J.-E. NYLUND. 1972. Blood reactions in vitro in crayfish against a fungal parasite, *Aphanomyces astaci. J. Invertebr. Pathol.* **19:** 94–106.

YOSHINO, T. P., AND T. C. CHENG. 1976. Fine structural localization of acid phosphatase in granulocytes of the pelecypod *Mercenaria mercenaria. Trans. Am. Fish. Soc.* **95:** 215–220.